Localization and Interactions of Teichoic Acid Synthetic Enzymes in Bacillus subtilis

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Table S1: Primers.

Primer	Primer sequence (5'-3')[a]	Description[b]	To Make[c]
GFP fusions			
TagB 1151 Fw	ATAGTACTCGAGTAATCCTCTATGCACCGAC	Fw *Xho*I	pSG5879
TagB 1151 Rev	TTGAGAAAGCTTGCTTATTAAATTTTCGATGAAATTC	Rev *Hin*dIII	pSG5879
TagF 1151 Fw	TACACGCTCGAGCCTACATGGAGAGATGATCAG	Fw *Xho*I	pSG5880
TagF 1151 Rev	TGACGTGAATTCTTCAGCTTTAAATACAGTATTAACAACC	Rev *Eco*RI	pSG5880
TagO 1151 Fw	ACTGCACTCGAGTTATTGCCAGCACGCTGG	Fw *Xho*I	pSG5881
TagO 1151 Rev	TCAGTCAAGCTTATTCCTTTTCACCAGCCGTTTATAAAAC	Rev *Hin*dIII	pSG5881
TagB 1151 Fw	ATAGTACTCGAGTAATCCTCTATGCACCGAC	Fw *Xho*I	pSG5879
Bacterial Two-Hybrid			
TagA B2H Fw	AGTTGATCTAGAGCAAACAGAGACTATTCACAATATTCC	Fw *Xba*I	pSG5882-pSG5883
TagA B2H Rev	ACTCGCAGGTACCTTAATCTGTTTTGTATGATCTTTTTCAGGC	Rev *Kpn*I	pSG5882-pSG5883
TagB B2H Fw	AGTTGATCTAGAGAAAATAAGATCACTACTGGCGAATTG	Fw *Xba*I	pSG5884-pSG5885
TagB B2H Rev	ACTCGCAGGTACCTTGCTTATT	Rev *Kpn*I	pSG5884-

	AAATTTTCGATGAAATTCAATA AATTTTGG		pSG5885
TagD B2H Fw	AGTTGATCTAGAGAAAAAAGT TATCACATATGGAACTTTTG	Fw *Xba*I	pSG5886-pSG5888
TagD B2H Rev	ACTCGCAGGTACCTTTAAACCA GCAATTTCCTCTTTG	Rev *Kpn*I	pSG5886-pSG5888
TagE B2H Fw	AGTTGATCTAGAGTCTTTACAT GCGGTGAGTGAATC	Fw *Xba*I	pSG5889-pSG5891
TagE B2H Rev	ACTCGCAGGTACCTTACTCTCT TTTATTTCCGTGACCCTC	Rev *Kpn*I	pSG5889-pSG5891
TagF B2H Fw	AGTTGATCTAGAGTCCTTAGTA GTTGACACTAATAAAAG	Fw *Xba*I	pSG5892-pSG5894
TagF B2H Rev	ACTGCAGGTACCTCTTCAGCTT TAAATACAGTATTAACAACC	Rev *Kpn*I	pSG5892-pSG5894
TagG B2H Fw	AGTTGATCTAGAGAATGATTTG TTGCGTATACTCA	Fw *Xba*I	pSG5895-pSG5896
TagG B2H Rev	ACTCGCAGGTACCTTAAGAAA GTCAACAAACTTGTCTCTG	Rev *Kpn*I	pSG5895-pSG5896
TagH B2H Fw	AGTTGATCTAGAGAAACTAAA AGTTTCGTTTCGAAATG	Fw *Xba*I	pSG5897-pSG5898
TagH B2H Rev	ACTCGCAGGTACCTTTTTCAAC ATCAAAGTCAGTGTATG	Rev *Kpn*I	pSG5897-pSG5898
TagO B2H Fw	AGTTGATCTAGAGCTTGACGA ACGCATGATTC	Fw *Xba*I	pSG5899-pSG5901
TagO B2H Rev	ACTCGCAGGTACCTTATTCCTT	Rev *Kpn*I	pSG5899-

	TTCACCAGCCG		pSG5901
Yeast Two-Hybrid			
mAD1ext	AACGGTCCGAACCTCATAAC		Gap repair
mBD1ext	GTCTCCGCTGACTAGGGCAC		Gap repair
mBD2ext	CGAGGGCTTATTCAGAAGCT		Gap repair
TagA Y2H Fw	GGAGGAGGAATTCATGCAAAC AGAGACT	Fw *Eco*RI	Gap repair
TagA Y2H Rev	GTTTTGGTCGACAATCTGTTTT GTATG	Rev *Sal*I	Gap repair
TagB Y2H Fw	GGATGGAATTCATGAAAATAA GATCACTA	Fw *Eco*RI	Gap repair
TagB Y2H Rev	TGTCATGTCGACGCTTATTAAA TTTTCG	Rev *Sal*I	Gap repair
TagD Y2H Fw	GCGTTTGGATCCATGAAAAAA GTTATCAC	Fw *Bam*HI	Gap repair
TagD Y2H Rev	GATCCTGTCGACTAAACCAGC AATTTCCT	Rev *Sal*I	Gap repair
TagF Y2H Fw	AAAAGAGGATCCATGTCCTTA GTAGTTGAC	Fw *Bam*HI	Gap repair
TagF Y2H Rev	TAACCTGTCGACTTCAGCTTTA AATACAG	Rev *Sal*I	Gap repair
TagG Y2H Fw	GGAAGAGAATTCATGAATGAT TTGTTGCG	Fw *Eco*RI	Gap repair
TagG Y2H Rev	CCTTACGTCGACAAGAAAGTC	Rev *Sal*I	Gap repair

	AACAAAC		
TagH Y2H Fw	GGAGATGGATCCATGAAACTA AAAGTTTCG	Fw *Bam*HI	Gap repair
TagH Y2H Rev	GCCTTGTCGACTTTCAACATCA AAGT	Rev *Sal*I	Gap repair
TagO Y2H Fw	GGAGACGAATTCATGCTTGAC GAACGCA	Fw *Eco*RI	Gap repair
TagO Y2H Rev	CCGGAGGTCGACATTCCTTTTC ACCAGCCG	Rev *Sal*I	Gap repair
MreC Y2H Fw	AGGTGTGAATTCATGCCGAAT AAGCGG	Fw *Eco*RI	pSG4561J-pSG4562J
MreC Y2H Rev	GAAGGAGGATCCTCACGATCC TTCCTC	Rev *Bam*HI	pSG4561J-pSG4562J
MreD Y2H Fw	GGAGGAGAATTCGTGAAACGT TTCCTT	Fw *Eco*RI	pSG4563J-pSG4564J
MreD Y2H Rev	ATAAAAGGATCCTTACTCATCT CTCAA	Rev *Bam*HI	pSG4563J-pSG4564J

[a] Restriction sites sequences are underlined.

[b] Restriction sites used, plus the direction of primers relative to the gene cloned abbreviations: Fw, Forward; Rev, Reverse.

[c] Use of the primers, for the cloning of the plasmids listed or for gap repair cloning in the yeast.

Table S2. Plasmids used for the two-hybrid experiments.

Plasmid	*Relevant Characteristics*	*Source/Construction*
Bacterial Two-Hybrid		
pUT18	P_{lac}-mcs-*cyaA*$^{675\text{-}1197}$ *bla*	(34)
pUT18c	P_{lac}-*cyaA*$^{675\text{-}1197}$-mcs *bla*	(34)
pUT18::zip	P_{lac}-*zip*-*cyaA*$^{675\text{-}1197}$ *bla*	(34)
pUT18c::zip	P_{lac}-*cyaA*$^{675\text{-}1197}$-*zip bla*	(34)
pKT25	P_{lac}-*cyaA*$^{1\text{-}732}$-mcs *kan*	(34)
pKT25::zip	P_{lac}-*cyaA*$^{1\text{-}732}$-*zip kan*	(34)
pSG5882	pKT25:: *tagA*	This work
pSG5883	pUT18:: *tagA*	This work
pSG5884	pKT25:: *tagB*	This work
pSG5885	pUT18:: *tagB*	This work
pSG5886	pKT25:: *tagD*	This work
pSG5887	pUT18:: *tagD*	This work
pSG5888	pUT18C:: *tagD*	This work
pSG5889	pKT25:: *tagE*	This work
pSG5890	pUT18:: *tagE*	This work
pSG5891	pUT18C:: *tagE*	This work
pSG5892	pKT25:: *tagF*	This work
pUT18	pUT18:: *tagF*	This work
pSG5894	pUT18C:: *tagF*	This work
pSG5895	pKT25:: *tagG*	This work

pSG5896	pUT18C:: *tagG*	This work
pSG5897	pKT25:: *tagH*	This work
pSG5898	pUT18C:: *tagH*	This work
pSG5899	pKT25:: *tagO*	This work
pSG5900	pUT18:: *tagO*	This work
pSG5901	pUT18C:: *tagO*	This work
pB11	pKT25:: *mreC*	(66)
pA11	pUT18:: *mreC*	(66)
pB12	pKT25:: *mreD*	(66)
pA12	pUT18C:: *mreD*	(66)
Yeast Two Hybrid		
pGAD-C1	P_{ADH1}-*GAL4* AD-mcs *LEU2 bla*	(30)
pGBDU-C1	P_{ADH1}-*GAL4* BD-mcs *URA3 bla*	(30)
pSG4561J	pGAD-C1:: *mreC*	This work
pSG4562J	pGBDU-C1:: *mreC*	This work
pSG4563J	pGAD-C1:: *mreD*	This work
pSG4564J	pGBDU-C1:: *mreD*	This work

[a] Resistance gene abbreviations as follows: bla, ampicillin; kan, kanamycin. Other abbreviations: mcs, multiple cloning site